1,094,567

PATENT SPECIFICATION

DRAWINGS ATTACHED

1,094,567

Date of Application and filing Complete Specification: Nov. 30, 1964.
No. 48596/64.
Application made in Japan (No. 35307) on June 23, 1964.
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Index at acceptance :—A5 E(1C3B1, 1C5C, 1C19)
Int. Cl.:— A 01 n 9/00

COMPLETE SPECIFICATION

Plant Disease Protective and Curative Compositions

ERRATA

SPECIFICATION No. 1,094,567

Page 1, line 43, *for* "deterioated" *read* "deteriorated"
Page 1, line 53, *for* "Kasugamycin" *read* "kasugamycin"
Page 1, line 76, *for* "the" *read* "its"
Page 1, line 77, *for* "Kasubamicin" *read* "kasugamicin"
Page 2, line 11, *for* "10 mg/cc." *read* "10 mcg/cc."
Page 2, line 15, *after* "after" *delete* "the"
Page 2, line 79, *for* "qypsum" *read* "gypsum"
Page 2, line 107, *for* "introduced" *read* "inoculated"
Page 3, line 21, *for* "evne" *read* "even"
Page 3, line 29, *for* "extracts" *read* "extract"
Page 3, line 31, *for* "The" *read* "the"
Page 4, line 18, *after* "purity" *delete* "hyphen"
Page 5, EXAMPLE 5, first column, line 6, *for* "mercutic" *read* "mercuric"
Page 8, line 19, *for* "preparations" *read* "preparation"
Page 8, line 29, *for* "where" *read* "were"
Page 9, EXAMPLE 12, first column, line 10, *for* "5000" *read* "500"
Page 10, line 14, *for* "agent" *read* "agents"

THE PATENT OFFICE
4th June 1968

octadecyl thiocyanate or blasticidin S, it is not deterioated in effect, but rather enhanced. Kasugamycin is harmless to men, animals and fishes. It is harmless to plants giving no toxic

curative effect while the latter is the protective effect. In the case of kasugamycin, the curative effect is stronger than the protective effect. Further, kasugamycin has excellent rain-

[*Price 4s. 6d.*]

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COMPLETE SPECIFICATION

Plant Disease Protective and Curative Compositions

We, ZAIDAN HOJIN BISEIBUTSU KAGAKU KENKYUKAI, a juridical foundation organized under the laws of Japan, of 403 Kamiosaki-Nakamaru, Shinagawa-ku, Tokyo, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to plant disease protective and curative compositions and provides a plant disease protective and curative composition comprising, as an active ingredient, kasugamycin or its acid addition salts, in an inert diluent or carrier.

In the accompanying drawings, Figures 1 and 2 show curves indicating respective preventive effects of kasugamycin and of other known chemicals against rice blast.

Conventionally, organic mercury compounds and blasticidin S have been used as preventive agents against rice blast. Since rice blast causes serious damage to rice, more effective agents have been required. When used for long periods, the organic mercury compounds tend to remain in the human body and hence the amount used thereof is required to be reduced. Further, blasticidin S has a strong toxicity, causing destruction of human mucous membrane. In this connection, the appearance of new active agents against rice blast has been desired.

Kasugamycin is a novel antibiotic discovered by Hamao Umezawa et al., the present inventors, which has shown excellent preventive effects against rice blast as compared with known agents not only in pot tests in a greenhouse but also in field tests. Even when used in admixture with conventional fungicides such as organic mercury compounds, higher alkylthiocyanates, particularly hexadecyl and octadecyl thiocyanate or blasticidin S, it is not deterioated in effect, but rather enhanced. Kasugamycin is harmless to men, animals and fishes. It is harmless to plants giving no toxic sign, even when used in an amount 100 times higher than the useful concentration. The properties of kasugamycin are described in detail in our copending Application No. 48595/64 (Serial No. 1094566) together with the production process thereof.

A test carried out by spraying an aqueous solution of Kasugamycin to young rice seedlings having three leaves on each main stem infected with *Piricularia oryzae* showed such results that kasugamycin showed effect of 55% inhibition of the leaf lesions at a concentration of 1.25 mcg/cc., 73% at 5 mcg/cc., and 100% at 10 mcg/cc. Even when sprayed at a concentration of 1 mg/cc., the solution showed no phytotoxicity. Kasugamycin displays its strong preventive effect against rice blast not only on leaves but also against that on the ears. For example, a test conducted by spraying the aqueous solution of kasugamycin at a concentration of 40 mcg/cc. to rice plants, two days after the infection of *Piricularia oryzae* showed that rice blast on the ears, stem-nodes and grains was completely controlled. Further, the spray of the solution at a concentration of 1.25 — 20 mcg/cc. obviously showed a preventive effect. On the other hand, a solution of blasticidin S showed phytotoxicity when the concentration was higher than 20 mcg/cc. and no complete prevention was observed. On the basis of the antimicrobial effects and low toxicity kasubamicin was tested for its effect against various plant diseases. According to tests in a green house, kasugamycin exhibited preventive effect against rust of wheat and against wild fire of tobacco plants.

Although its action mechanism is not clear, kasugamycin shows stronger preventive effect when a solution thereof is sprayed on plants after the infection than before the infection. Ordinarily, the former effect is called the curative effect while the latter is the protective effect. In the case of kasugamycin, the curative effect is stronger than the protective effect. Further, kasugamycin has excellent rain-

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resistant properties. For example, in a test carried out by spraying solutions of kasugamycin to plants two days after the infection of *Piricularia oryzae*, water shower was given to the plants 30 minutes after the spray and then after 7 days in a green house, the number of enlarged lesion were calculated. In the case of kasugamycin at a concentration of 10 mcg/cc., the number of enlarged lesion per each leaf was 2.2, whereas that was 4.8 in the case of blasticidin S at 10 mg/cc. (control showed 36.9). In order to know preventive effects against damage due to rice blast, it is also useful to investigate the effect on outcome of new healthy leaves after the infection and the treatment. In this test kasugamycin showed excellent results as compared with blasticidin S.

Kasugamycin or its acid addition salts, either in pure or crude form, display preventive effect. Even a cultured liquid containing kasugamycin, either as such or in a condensed or dried state, exhibit preventive effects as well. Simultaneously with kasugamycin, *Streptomyces kasugaensis* (ATCC 15714 and 15715) sometimes produces aureothricin, thiolutin and a pentaene antifungal substance. Usually these antibiotics in the cultured broth show no harmful effect at the dilution at which kasugamycin is effectively used.

In accordance with the present invention, it is possble to use pure or crude kasugamycin or its acid addition salts, a kasugamycin-containing culture-liquid, the dried culture-liquid or a crude extract of kasugamycin in an inert diluent or carrier for the prevention and cure of plant diseases. In the case of using a kasugamycin-containing culture-liquid, its filtrate or dried culture-liquid, or kasugamycin-containing extract is used, other incorporated antibiotics are removed, if necessary.

Kasugamycin can be used in combination with conventional mercury fungicidal preparations, blasticidin S or alkylthiocyanates particularly hexadecyl and octadecylthiocyanate. Such combined use can serve to make the antimicrobial spectrum of kasugamycin broader. The present invention involves the combined agents as well.

Thus the present invention provides a composition which is effective against rice blast and which contains 1—1000 parts by weight of kasugamycin or its acid addition salts and 1—20 parts by weight of a phenyl mercury compound such as phenyl mercuric iodide or phenyl mercuric acetate, and a carrier.

The present invention further provides a composition which is effective against rice blast and which contains a mixture of 1—1000 parts by weight of kasugamycin or its acid addition salts and 1—1000 parts by weight of blasticidin S, and a carrier.

The present invention further provides a composition which contains 1—1000 parts by weight of kasugamycin or its acid addition salts in admixture with 1—1000 parts by weight of alkylthiocyanate such as hexadecyl and octadecylthiocyanate, and a carrier thereof.

According to the present invention, a composition containing kasugamycin can be formed, by the conventional process applied to preparation of ordinary agricultural pesticides, into a suitable form such as for example dust, liquid, emulsiable liquid or wettable powder. A dust formulation is obtained by mixing kasugamycin or a kasugamycin-containing material with a solid carrier such as calcium carbonate, qypsum, clay, talc, sieklite, vermiculite or diatomaceous earth; a wettable powder by mixing the same with, for example, a solid carrier and a surface active agent; a liquid formulation by adding the same to water or methanol or organic solvents-containing water; and an emulsifiable liquid formulation by emulsifying the same with the addition of a surface active agent. If necessary, a spreader and a stabilizer may further be added. Kasugamycin is stable under neutral, weakly acidic and acidic conditions but is less stable under alkaline conditions and hence is desirably formulated into a neutral or acidic state. Further, kasugamycin is basic, so that it is necessary to take into consideration the case where it is absorbed with an acidic solid. The dust, liquid, emulsifiable liquid and wettable powder formulations can be prepared in combination with conventional fungicides.

Method I *Test on curative effect.*

The test was carried out according to the pot test method. The rice plant tested was the mongolian variety seedlings having three leaves on each main stem. 20 rice grains just before germination were sowed in each of pots of 9 cm. in diameter. When there were three leaves on each main stem a spore suspension of *Piricularia oryzae* was introduced to the seedlings. The inoculated seedlings were kept in a moist chamber for 20 hours and then a solution of each chemical tested was sprayed thereto under a pressure of 0.5 kg/cm^2 in an amount of 4 ml/pot. To each chemical, 100 mcg/cc. of Newcol-560 was added as a spreader. The seedlings were allowed to stand in a green house for 5 — 7 days and then the number of enlarged lesions per 10 leaves was calculated. The curative value was evaluated by the following formula:

$$\text{C.V.} = \frac{\text{Number of lesions untreated} - \text{number of lesions treated}}{\text{Number of lesions untreated}} \times 100$$

Method (2): *Pot test on protective effect*

The procedures were the same as in the method (1), but rice seedlings were treated with chemicals before the inoculation of *Piricularia oryzae.*

The protective value was similarly evaluated as the curative value.

EXAMPLE 1:

In this example, the method (1) was adopted, a filtrate of cultured liquid *Streptomyces kasugaensis* which contained about 200 mcg/cc. of kasugamycin was extracted with butanol to remove aureothricin, thiolutin and a pentaene fungicidal substance. The filtrate was diluted with distilled water 2, 4, 8 and 16 times, and, to each of the diluted filtrate was added 100 mcg/cc. of Newcol-560 as a spreader. Each agent thus prepared was sprayed to seedlings having three leaves on their main stems.

Piricularia oryzae.

The results are shown in the following table:

Agents sprayed	Number of enlarged lesions per 10 leaves
Kasugamycin culture filtrate	0
Kasugamycin culture filtrate diluted 2 times	0
Kasugamycin culture filtrate diluted 4 times	0
Kasugamycin culture filtrate diluted 8 times	0.9
Kasugamycin culture filtrate diluted 16 times	3.0
Control	84.5

It is clear from the above table, that the present agent, evne when diluted 16 times, can inhibit the progress of infection.

EXAMPLE 2:

In this example, the method (1) was adopted, a filtrate of cultured liquid of *Streptomyces kasugaensis* which contained 100 mcg/cc. of kasugamycin (the filtrate further contained about 10 mcg/cc. of aureothricin and thiolutin), an aqueous solution of extracts of a culture-liquid of *Streptomyces kasugaensis* (The said extract was a powder obtained by adding an active carbon to the cultured-liquid at 0.5% and pH 2.0, removing the active carbon by filtration, neutralizing the resulting filtrate, adding thereto 2.0% of active carbon to adsorb kasugamycin, eluting the kasugamycin with 80% aqueous methanol at pH 7.0 and then concentrating the same under reduced pressure followed by drying), and an aqueous solution of kasugamycin hydrochloride were tested, respectively. The results obtained were as follows:

Agents sprayed	Curative value
Filtrate, diluted 2 times	100%
„ „ 4 „	„
„ „ 16 „	„
„ „ 64 „	97.8%
Extract, 8 mg/cc.	100%
„ 4 „	„
„ 1 „	„
„ 0.5 „	98.0%
„ 0.25 „	88.5%
Kasugamycin hydrochloride 30 mcg/cc.	100%
„ „ 15 „	„
„ „ 3.75 „	98.2%
„ „ 1.0 „	82.2%

EXAMPLE 3:
The same agents as in example 2 were tested according to the method (2) to obtain the following results:

Agents sprayed	Protective effect value
Filtrate, diluted 2 times	100%
„ „ 8 „	„
„ „ 16 „	„
Extract, 4 mg./cc.	„
„ 2 „	99.5%
„ 1 „	93.7%
Kasugamycin hydrochloride 30 mcg/cc.	100%
„ „ 15 „	97.8%
„ „ 7.5 „	96.7%
„ „ 3.75 „	89.0%

EXAMPLE 4:
To 3 g. of a crude powder of 8% purity kasugamycin extracted from a filtrate of cultured liquid of *Streptomyces kasugaensis* according to ion exchange resin process, 60 g. of sieklite, 34 g. of talc, and 2 g. of vitasil (trade name of pulverised silicic acid) were added to form a dust formulation. The dust formulation thus prepared was dusted in an amount of 200 mg/pot. Further, wettable powder formulation was prepared by adding to 20 g. of crude powder of 8% purity-kasugamycin, 75 g. of Tolite MT-S (trade name of diatomaceous earth), 2 g. of Newcol-560 (trade name of surface active agent nonylphenol polyoxyethylene oxide) and 3 g. of Toyolignin CP

(trade name of lignin sulphonate). The formulation was diluted to 500 times with water and was then sprayed in an amount of 4 ml./pot. Each test was carried out according to the method (1) and showed the curative value within the range of from 90 to 100%.

EXAMPLE 5:

Kasugamycin was mixed with mercury preparations and the resulting chemical mixtures were compared in preventive effect with individual chemicals to obtain the following results (the method (1) was adopted):

Agents and concentration	Number of enlarged lesions per leaf
Kasugamycin 30 mcg/cc.	0
" 15 "	0.3
" 7.5 "	2.3
" 3.75 "	4.8
Kasugamycin 15 mcg/cc + phenyl mercutic iodide 20 mcg/cc.	0
Kasugamycin 15 mcg/cc + phenyl mercuric iodide 10 mcg/cc.	0.2
Kasugamycin 15 mcg/cc + phenyl mercuric iodide 5 mcg/cc.	2.1
Kasugamycin 7.5 mcg/cc + phenyl mercuric iodide 20 mcg/cc.	0
Kasugamycin 7.5 mcg/cc + phenyl mercuric iodide 10 mcg/cc.	0.2
Kasugamycin 7.5 mcg/cc + phenyl mercuric iodide 5 mcg/cc.	2.1
Kasugamycin 3.75 mcg/cc + phenyl mercuric iodide 20 mcg/cc.	0.1
Kasugamycin 3.75 mcg/cc + phenyl mercuric iodide 10 mcg/cc.	0.2
Kasugamycin 3.75 mcg/cc + phenyl mercuric iodide 5 mcg/cc.	2.9
Phenyl mercuric iodide 40 mcg/cc	16.6
" " " 20 "	20.2
" " " 10 "	20.8
" " " 5 "	18.7
Kasugamycin 15 mcg/cc + phenyl mercuric acetate 10 mcg/cc.	0
Phenyl mercuric acetate 20 mcg/cc.	19.5
Control	20.4

The above results show that the mercury preparations can be mixed with kasugamycin without reducing the activity of either. The mixture is in fact synergistic as far as the fungicidal properties are concerned.

EXAMPLE 6:

The same chemicals as in Example 5 were tested according to the method (2), i.e. *Piricularia oryzae* was inoculated to plants after the application of agents to obtain the following results:

Agents sprayed	Number of enlarged lesions per leaf
Kasugamycin 15 mcg/cc.	3.9
„ 7.5 „	8.3
Phenyl mercuric iodide 10 mcg/cc.	1.1
„ „ „ 5 „	3.0
Phenyl mercuric acetate 20 mcg/cc.	3.4
Kasugamycin 15 mcg/cc + phenyl mercuric iodide 10 mcg/cc.	0
Kasugamycin 15 mcg/cc + phenyl mercuric iodide 5 mcg/cc.	0.6
Kasugamycin 7.5 mcg/cc + phenyl mercuric iodide 5 mcg/cc.	0.8
Kasugamycin 15 mcg/cc + phenyl mercuric iodide 20 mcg/cc.	0.9
Control	20.0

The above results show that the mixing of the two agents increases the protective effect.

EXAMPLE 7:

100 mcg/cc of NNP (Newcol-560) was added as a spreader to a liquid containing kasugamycin in a concentration of 40 mcg/cc obtained by extracting a filtrate of cultured liquid of *Streptomyces kasugaensis* with butanol to remove aureothricin, thiolutin and a pentaene antifungal substance. The chemical thus prepared was subjected to a field test. The test was carried out in the field of Agricultural Experiment Station of Hokkoh Chemical Industry Co. at Atsugi in Kanagawa Prefecture. In this field, rice plant was inoculated with diseased rice leaves in order to cause vigorous infection of *Piricularia oryzae*. Four days after the inoculation, i.e. when the appearance of diseased spots was recognized, 20 cc. per 0.6 m^2 of the above-mentioned kasugamycin-containing agent was sprayed. The spray was conducted 1 to 3 times. For comparison, 20 mcg/cc. of phenyl mercuric iodide and 40, 20 and 10 mcg/cc. of blasticidin S were sprayed, respectively, in an amount of 20 cc. per 0.6m^2 to respective plot. At 7,9,11, 13,15,19 and 23rd day after the inoculation with diseased leaves, the area of lesions in each plot was calculated and the results were showed in Figs. 1 and 2 of the accompanying drawings. In each graph, the vertical axis shows the percentage of diseased lesion area; the horizontal axis shows the number of days; curve A indicates a control (non-treated; curve B the results of 20 mcg/cc mercury chemical; curve C, 10 mcg/cc. blasticidin S; curve D, 20 mcg/cc. blasticidin S; curve E, 40 mcg/cc. blasticidin S; and curve F, kasugamycin. Fig. 2 shows the results of test in which the first spray was carried on the fourth day after the inoculation, the second spray on the ninth day, and the third spray on the eleventh day. It was noticed that the rice plants treated with kasugamycin were least in the number of lesions and the growth of plants was markedly strong.

EXAMPLE 8

In this example, there were used kasugamycin hydrochloride, a crude kasugamycin of 1.5% purity and a filtrate of cultured liquid of *Streptomyces kasugaensis* containing 400 mcg/cc. of kasugamycin at 1.5 and 30 mcg/cc. of aureothricin. They were diluted with water to obtain solutions containing kasugamycin at concentrations of 80 mcg/cc, 40 mcg/cc, 20 mcg/cc and 10 mcg/cc, respectively. To each solution, Newcol-560 was added at 100 mcg/cc. In the same manners as in Example 7, the agents thus prepared were sprayed in the field test. For comparison, solutions of blasticidin S at concentrations of 20 mcg/cc. and 10 mcg/cc were sprayed. One month after the spray the solutions containing kasugamycin at

80 mcg/cc gave the best results and seedlings sprayed therewith grew like non-infected seedlings. Furthermore seedlings sprayed with the filtrate of cultured liquid showed stronger growth than non-infected seedlings. Among the infected seedlings, almost all of untreated seedlings were killed. Those sprayed with kasugamycin were stronger in growth than those sprayed with blasticidin S.

EXAMPLE 9:

Kasugamycin hydrochloride and its mixture with aureothricin were tested according to the method (1) to obtain the following results:

Agents sprayed	Number of enlarged lesions per 10 leaves
Kasugamycin 20 mcg/cc.	0
„ 5 „	7.4
„ 1.25 „	50.4
Kasugamycin 20 mcg/cc + aureothricin 5 mcg/cc.	2.7
Kasugamycin 5 mcg/cc + aureothricin 5 mcg/cc.	10.2
Kasugamycin 1.25 mcg/cc + aureothricin 5 mcg/cc.	25.6
Blasticidin S 30 mcg/cc.	8.4
Control	102.4

It was observed that aureothricin enhanced the actions of low concentration kasugamycin without lowering its preventive effects. That the presence of a slight amount of aureothricin or thiolutin accelerates the growth of plants was already known. This example showed that the presence of aureothricin at the concentration causing no phytotoxicity does not interfere with the effect of kasugamycin.

EXAMPLE 10:

Kasugamycin hydrochloride, blasticidin S and a mixture of both in equal amounts were prepared and their aqueous solutions were tested according to the method (1) to obtain the following results:

Agents sprayed	Curative value
Kasugamycin 30 mcg/cc.	100%
„ 15 „	96.5%
„ 7.5 „	92.1%
„ 3.75 „	63.0%
Blasticidin S 30 mcg/cc.	98.5%
„ 20 „	98.0%
„ 10 „	89.0%
Mixture 30 mcg/cc.	100%
„ 15 „	97.0%
„ 7.5 „	86.0%
„ 3.75 „	73.2%

EXAMPLE 11:

Test for examination of preventive effect in field.

Method: In a vinyl house, rice seedlings were inoculated with rice leaves which were severely infected with *Piricularia oryzae*. Two days after the inoculation, dust and wettable powder formulations of each agent of kasugamycin, phenyl mercuric iodide and kasugamycin + phenyl mercuric iodide were sprayed one week thereafter, the same agents were again sprayed, 12, 17 and 25 days after the first spray, the areas of leaves which were killed due to *Piricularia oryzae* were investigated to obtain the following results:

Agents and concentration		Killed area (%) after 12 days	17 days	25 days
None		77.9%	Completely killed	Completely killed
Wettable powder formulation:				
Kasugamycin	20 ppm.	8.1	34	50
Phenyl mercuric iodide	20 ppm. (as Hg)	19.7	58	80
Kasugamycin + phenyl mercuric iodide	10 ppm. 10 ppm. (as Hg)	6.6	26	30
Dust formulation:				
Kasugamycin	0.2%	17.9	55	63
Phenyl mercuric iodide	0.2% (as Hg)	24.5	62	45
Kasugamycin + phenyl mercuric iodide	0.1% 0.1% (as Hg)	6.6	24	30

The above results show that, in the preventive test in field also, the mixture of kasugamycin with mercury preparations exhibits synergistic effect.

EXAMPLE 12:

Kasugamycin was mixed with alkylthiocyanates (a mixture of hexadecyl- and octadecylthiocyanate) in the ratio of the former 15—35 and the latter 85—65 percentage at various concentrations in aqueous solutions and the preventive effects of the resulting mixtures were tested according to the method (1) and where compared with the effects of individual agents.

Test results were as follows:

Agents and concentration	Number of enlarged lesions per 10 leaves		
	Plot A	Plot B	Average
Kasugamycin 15 mcg/cc.	0.2	0.3	0.3
„ 7.5 „	2.1	2.4	2.3
„ 3.75 „	3.5	6.1	4.8
Alkylthiocyanate 1000 mcg/cc + kasugamycin 15 mcg/cc.	0	0	0
Alkylthiocyanate 1000 mcg/cc + kasugamycin 7.5 mcg/cc.	0.6	0.8	0.7
Alkylthiocyanate 1000 mcg/cc + kasugamycin 3.75 mcg/cc.	0.2	1.1	0.7
Alkylthiocyanate 5000 mcg/cc + kasugamycin 15 mcg/cc.	0	0	0
Alkylthiocyanate 500 mcg/cc + kasugamycin 7.5 mcg/cc.	0.5	0.7	0.6
Alkylthiocyanate 500 mcg/cc + kasugamycin 3.75 mcg/cc.	1.8	2.4	2.1
Alkylthiocyanate 1000 mcg/cc.	13.7	19.2	16.5
Alkylthiocyanate 500 mcg/cc.	15.1	20.2	17.7
Blasticidin S 30 mcg/cc.	0	0	0
Control	13.2	20.1	16.7

The above results indicate that respective curative effects of alkylthiocyanates and kasugamycin are increased when the two are used in admixture, and that the mixed agent is more effective than twice the amount of individual agent. Thus, the agents show synergistic effect when used in admixture.

EXAMPLE 13:

The same agents as in example 12 were tested according to the method (2).

The test results were as follows:

Agents and concentration	Number of enlarged lesions per leaf			
	Sect. a	Sect. b	Sect. c	Average
Kasugamycin 15 mcg/cc.	2.7	3.9	4.8	3.7
„ 7.5 „	5.0	4.2	6.4	5.2
„ 3.75 „	8.2	8.7	10.1	9.0
Alkylthiocyanate 100 g/cc.	0	0.17	0.43	0.20
„ 50 „	0	1.50	2.00	1.00
Alkylthiocyanate 100 mcg/cc + kasugamycin 15 mcg/cc.	0	0	0	0
Alkylthiocyanate 100 mcg/cc + kasugamycin 7.5 mcg/cc.	0	0	0.03	0.86
Alkylthiocyanate 100 mcg/cc + kasugamycin 3.75 mcg/cc.	0	0	0	0
Alkylthiocyanate 50 mcg/cc + kasugamycin 15 mcg/cc.	0	0	0	0
Alkylthiocyanate 50 mcg/cc + kasugamycin 7.5 mcg/cc.	0	0	0.29	0
Alkylthiocyanate 50 mcg/cc + kasugamycin 3.75 mcg/cc.	0	0	0.59	0.20
Control	12.57	12.15	14.60	13.11

The above results indicate that the mixed agents are higher in preventive effect than twice the amount of individual agents and obviously show synergistic effects.

EXAMPLE 14:

Field test I

Method: Rice seedlings "Norin No. 21" having four or five leaves on each main stem and cultivated in nursery state had *Piricularia oryzae* inoculated into them using diseased leaves as an inoculation source. After recognizing that the seedlings had been attacked the same agent as in example 12 were sprayed 3 times and the diseased state of the seedlings was investigated 4 times before and after each spray.

Date of inoculation: May 10, 1964
Date of spray: 1. May 18, 1964
2. May 25, 1964
3. June 1, 1964

The results were as follows:

Agents and concentration	Area of lesions (%)			
	May 17	May 24	May 31	June 7
Kasugamycin 60 mcg/cc.	2	4	8	20
» 30 »	3	5	23	39
» 15 »	2	9	28	55
» 7.5 »	4	12	46	80
Kasugamycin 30 mcg/cc + alkylthiocyanate 1000 mcg/cc.	2	5	10	18
Kasugamycin 30 mcg/cc + alkylthiocyanate 500 mcg/cc.	3	8	11	21
Kasugamycin 30 mcg/cc + alkylthiocyanate 250 mcg/cc.	4	6	11	29
Kasugamycin 15 mcg/cc + alkylthiocyanate 1000 mcg/cc	2	9	10	22
Kasugamycin 15 mcg/cc + alkylthiocyanate 500 mcg/cc.	2	8	17	31
Kasugamycin 15 mcg/cc + alkylthiocyanate 250 mcg/cc.	2	8	23	41
Kasugamycin 7.5 mcg/cc + alkylthiocyanate 1000 mcg/cc.	2	5	15	38
Kasugamycin 7.5 mcg/cc + alkylthiocyanate 500 mcg/cc.	3	10	20	56
Kasugamycin 7.5 mcg/cc + alkylthiocyanate 250 mcg/cc.	3	12	21	33
Alkylthiocyanate 1000 mcg/cc.	2	6	24	42
» 500 mcg/cc.	2	13	35	64
» 250 mcg/cc.	3	14	52	83
Phenyl mercuric iodide 20 mcg/cc.	2	14	56	72
Blasticidin S 30 mcg/cc.	4	17	35	61
Control	2	28	74	95

Field test II

Method: In the same manners as in Field test I, rice seedlings were grown in a nursery in a green-house and diseased leaves were distributed on May 16, 1964 in the nursery to inoculate *Piricularia oryzae* to the seedlings. Two days after the inoculation, each chemical in the form of a dust formulation of kasugamycin, alkylthiocyanate (a mixture of hexadecyl- and octadecyl thiocyanate) and kasugamycin + the said alkylthiocyanate was sprayed two times, 12, 17 and 25 days after the first spray, the areas of leaves killed due to *Piricularia oryzae* were investigated. The results were as follows:

Chemicals	Concentration	Area of killed leaves (%) after 12 days	17 days	25 days
None	—	77.9	Completely	Killed
Alkylthiocyanate	4.0%	34.5	60	85
Kasugamycin	0.2%	27.9	55	68
I Kasugamycin + alkylthiocyanate	0.1% 2.0%	12.1	27	41
Kasugamycin	0.1%	39.5	81	94
II Kasugamycin + alkylthiocyanate	0.05% 2.0%	14.7	33	51

It is clear that a synergistic effect is attained by mixing the two agents.

WHAT WE CLAIM IS:—

1. A plant disease protective and curative composition comprising, as an active ingredient, kasugamycin or its acid addition salts in an inert diluent or carrier.

2. A composition as claimed in claim 1 including other fungicides.

3. A composition as claimed in claim 2, which is effective against rice blast and which contains 1—1000 parts by weight of kasugamycin or its acid addition salts and 1—20 parts by weight of a phenyl mercury compound such as phenyl mercuric iodide or phenyl mercuric acetate, and a carrier.

4. A composition as claimed in claim 2 which is effective against rice blast and which contains a mixture of 1—1000 parts by weight of kasugamycin or its acid addition salts and 1—100 parts by weight of blasticidin S, and a carrier.

5. A composition as claimed in claim 2, which contains 1—1000 parts by weight of kasugamycin or its acid addition salts in admixture with 1—100 parts by weight of alkylthiocyanate such as hexadecyl and octadecylthiocyanate, and a carrier thereof.

6. A composition according to claim 1 substantially as herein described with reference to any one of the Examples.

7. A method of inhibiting plant diseases which comprises applying to the plant a composition as claimed in any of the preceding claims.

8. A method as claimed in claim 7 and substantially as herein decribed with reference to the Examples.

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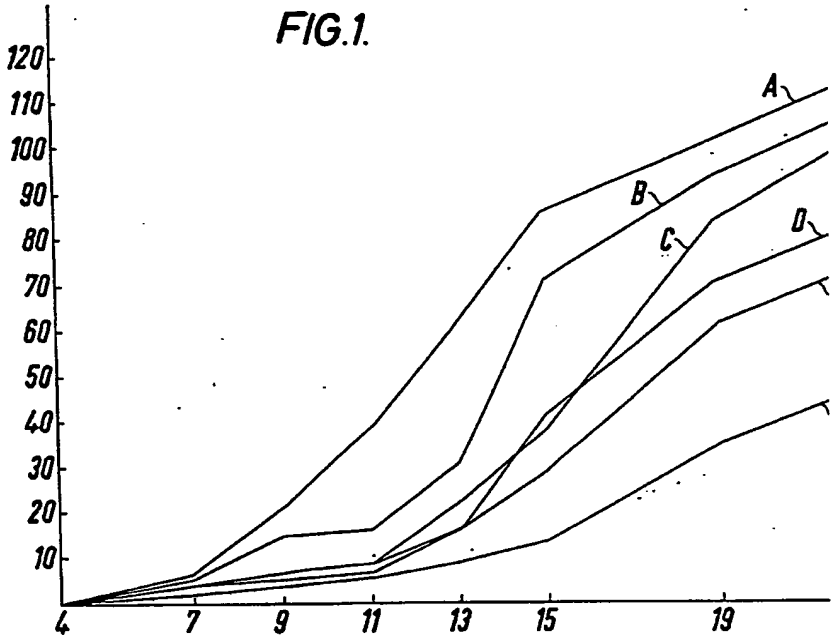
FIG.1.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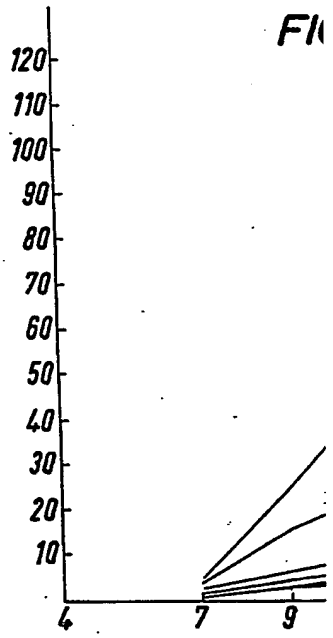
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1094567 COMPLETE SPECIFICATION
1 SHEET *This drawing is a reproduction of the Original on a reduced scale*

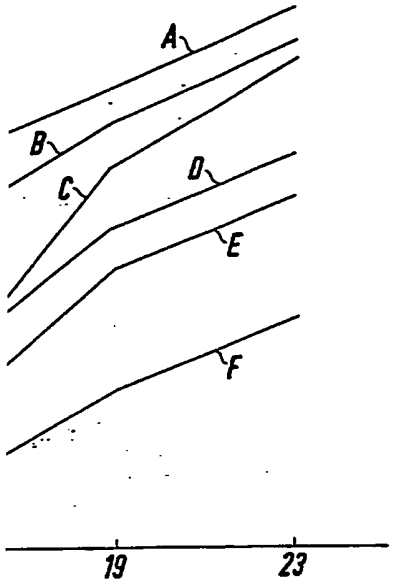

FIG.2.

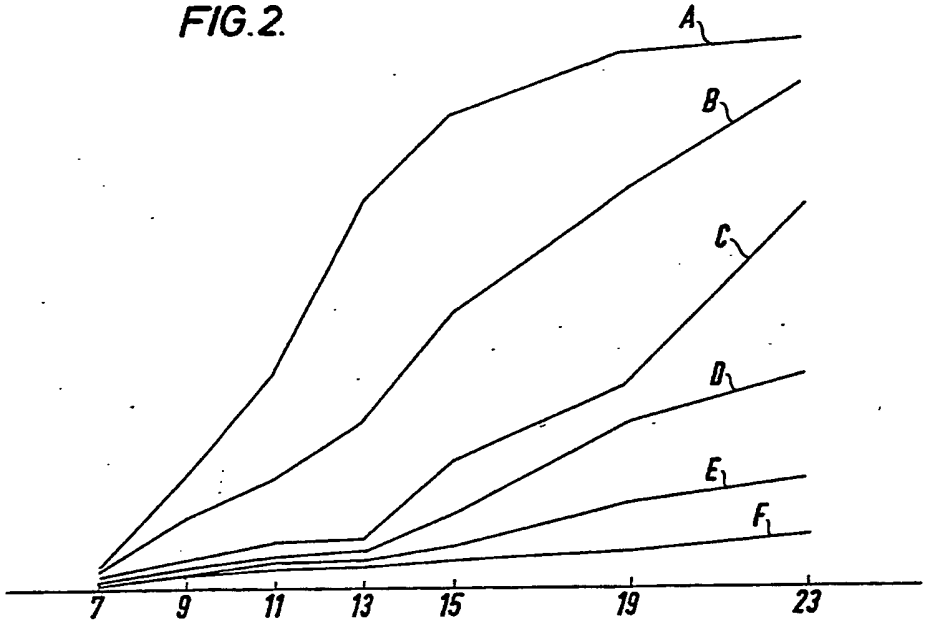

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FIG.1.

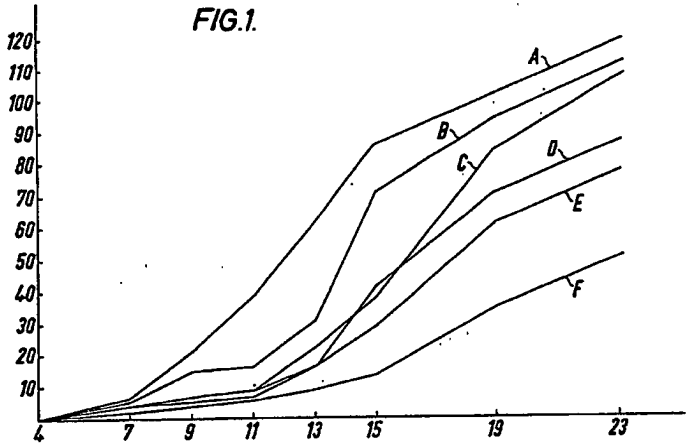

FIG.2.